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PATENTS
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Derek P. Freyberg

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4/8/99

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

John J. Nestor et al.

:

App. No.: 08/453,223

: Art Unit: 1611

Filed: May 30, 1995

: Examiner: Mark L. Berch

For: 2-(2-AMINO-1,6-DIHYDRO-6-OXO-PURIN-9-YL)METHOXY-
1,3-PROPANEDIOL DERIVATIVE

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF YEUN-KWEI HAN

I, Yeun-Kwei Han, a citizen of the United States residing in Louisville, Colorado, declare as follows:

I received a BS in Chemistry from Tamkang University, Taipei, Taiwan, in 1966, and a PhD in Chemistry from Northern Illinois University, DeKalb, Illinois, in 1978. From 1977 to 1979, I held a postdoctoral fellowship in synthetic organic chemistry at Ohio State University with Professor Leo A. Paquette. I have been employed as a chemist at Syntex Chemicals, Inc., now Roche Colorado Corporation, since August 1979 (in the Boulder Technology Center since May 1988) as a process chemist, and am now a Principal Research Chemist in the

Boulder Technology Center. I have worked extensively in the synthesis of ganciclovir and its esters and related nucleosides.

I, or persons working with me, have conducted the following experiments in an attempt to prepare crystalline ganciclovir bis(L-valinate) hydrochloride (GBVH).

Preparation of ganciclovir bis(CBZ-L-valinate)

10.07 g ganciclovir, 0.4 g 4-dimethylaminopyridine, and 80 mL *N,N'*-dimethylformamide were charged to a reactor. 28.8 g CBZ-L-valine-*N*-carboxyanhydride was dissolved in 100 mL ethyl acetate, and added dropwise to the ganciclovir solution over 140 minutes at room temperature. The resulting mixture was stirred overnight at room temperature. A homogeneous solution was obtained; and high performance liquid chromatography showed that the reaction was complete, with better than 97% product. The reaction was quenched with 100 mL water and diluted with 150 mL ethyl acetate. The aqueous and organic layers were separated; and the organic layer was washed with 100 mL 2% aqueous acetic acid, with a mixture of 50 mL saturated aqueous NaHCO₃ and 50 mL water, and three times with 100 mL water. The washed organic layer was concentrated to dryness on a rotary evaporator, forming an oil, then the product redissolved in 100 mL ethyl acetate and again concentrated to dryness. 100 mL hexane was added to dissolve the product, which was then allowed to stand overnight at room temperature. The product slowly crystallized, and was filtered and washed with hexane, then dried under vacuum at room temperature to yield 28.54 g crude ganciclovir bis(CBZ-L-valinate) product, with a purity exceeding 97% by area normalized HPLC.

Preparation of GBVH

0.5 g palladium hydroxide on carbon and 15 mL methanol were added to a 150 mL pressure reaction vessel connected to a hydrogen gas system. The vessel was purged three times with nitrogen, then three times with hydrogen, then left stirring under 35 psig hydrogen at room temperature. 8.60 g ganciclovir bis(CBZ-L-valinate), prepared as described above, 40 mL methanol, and 2.35 g 37% hydrochloric acid were added to a beaker containing a magnetic stirrer, and stirred to dissolve the solids. The reaction vessel was purged three times with

nitrogen, then the solution containing the ganciclovir bis(CBZ-valinate) was added. The vessel was purged three times with nitrogen, three times with hydrogen, and then held at 35 psig hydrogen with stirring. The vessel was heated to 45°C for 70 minutes, then vented and purged three times with hydrogen. After a further 70 minutes, a sample was taken for analysis, and the reaction vessel was re-purged and held at 35 psig hydrogen pressure with stirring. Thin layer chromatography of the reaction mixture indicated disappearance of the starting material. After a total of 5 hours reaction time, the vessel was cooled and the reaction mixture removed, filtered, and the solvents evaporated on a rotary evaporator under vacuum to give 6.27 g (100.3% crude yield) of white foamy solids. The GBVH had a purity of approximately 92.7% by area normalized HPLC; and mass spectrometry, UV spectroscopy, and NMR analyses were consistent with GBVH.

Examination of the GBVH

Because the GBVH prepared as described above had a shiny surface appearance, a sample was examined microscopically using both brightfield and polarized light illumination. The surface morphology in brightfield illumination indicated an amorphous material with no defined crystal faces. Polarized light microscopy showed a dull white material with no extinction evident during 360° rotation of the stage; however, some homogenized slivers showed extinction on rotation, indicating possible polycrystallinity.

To determine possible crystallinity, a sample was subjected to X-ray powder diffraction analysis, using a Scintag Model X1 powder diffractometer with Cu K α radiation, scanning a 2 θ angle from 2° to 40°. The X-ray diffraction spectrum indicated only a broad diffuse hump centered at approximately 24° 2 θ , with a full width at half-height of approximately 12°, and no sharp peaks. The X-ray diffraction pattern is characteristic of an amorphous material, indicating lack of crystallinity of the GBVH. A copy of the X-ray diffraction spectrum is attached as the Exhibit to this Declaration.

Attempts at recrystallization of GBVH

1. 4.1 g GBVH was dissolved in approximately 4 mL water, and the mixture warmed to approximately 40°C to dissolve the solids, then

heated to 60°C. 13 mL isopropanol was added rapidly, followed by another 25 mL isopropanol added dropwise. By the end of the addition, the solution had become slightly cloudy; and a further addition of 25 mL isopropanol caused no solid formation, though the solution remained cloudy. The solution was cooled slowly to 0 - 5°C, ultimately in an ice bath, resulting in the formation of an oily product. Attempts to break the oil by scraping the walls of the flask with a spatula were unsuccessful. A sample of the solid material was isolated and examined microscopically under brightfield conditions. The material was seen to be an oil, with clear evidence of globules, and no evidence of crystal formation.

2. The material from the attempt above was concentrated to dryness on a rotary evaporator under 10 mmHg and a temperature up to approximately 40°C. 1.55 g water was added, and the material was stirred at 40°C to dissolve the solids. 13 mL isopropanol was added, with solution becoming cloudy; and the solution heated to 60°C. Another 25 mL isopropanol was added dropwise, resulting in formation of an oil. The material was slowly cooled to room temperature, then cooled to 0 - 5°C in an ice bath. Attempts to break the oil by scraping the walls of the flask with a spatula, followed by stirring at 0 - 5°C for several hours, were again unsuccessful, though some fine particles were broken from the mass. A sample of the solid material was isolated and examined microscopically under brightfield and polarized light conditions. Under brightfield conditions, the material was seen to be an amorphous powder, with no evidence of crystallinity; and under polarized light conditions, no evidence of anisotropy was seen, indicating that the GBVH was amorphous.

Conclusion

Ganciclovir bis(L-valinate) hydrochloride as prepared is non-crystalline, and attempts to prepare it in crystalline form using conditions calculated to cause crystallization of the material [which conditions lead to ready crystallization of ganciclovir mono(L-valinate) hydrochloride] have been unsuccessful. I believe that it is not possible to prepare ganciclovir bis(L-valinate) hydrochloride in crystalline form without undue experimentation, if at all.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under 18 USC 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4/16/99



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